Volume 2, Issue 6, June - 2024 **ISSN(E):** 2938-3773

Web of Discoveries: Journal of Analysis and Inventions
webofjournals.com/index.php/3

MODERN TYPES OF IMMUNOENZYME ANALYSIS METHODS OLD PROBLEMS

Kudratova Zebo Erkinovna
PhD, Associate Professor of the Department of Clinical Laboratory Diagnostics with the Course of PGD Clinical and Laboratory Diagnostics;

Tamila Abdufattoevna Umarova
Department of Clinical Laboratory Diagnostics with the Course of PGD Clinical and Laboratory Diagnostics;

Sirojeddiova Sanobar
Clinical Resident of the Department of Clinical Laboratory Diagnostics with the Course of PGD Clinical and Laboratory Diagnostics
Samarkand State Medical University Samarkand, Uzbekistan

Abstract
The basis of any ELISA variant is the determination of enzymatic reaction products in the examination of tested samples in comparison with negative and positive controls. The method has high sensitivity and specificity, which is 90%.

Keywords: immunoenzymatic method, antibodies, antigen, concentration, affinity, immunocomplexes.

Introduction

The primary process in immunoassay (or immunoassay) is the stage of "recognition" of the analyzed compound by an antibody specific to it. Since the process of formation of immunochemical complexes occurs in a strictly quantitative relationship due to affinity, concentrations of components and reaction conditions, it is sufficient to determine the initial concentration of the analyzed compound to quantify the immune complexes formed. For this assessment, either direct determination of the concentration of immunocomplexes formed (type 1) or quantification of the remaining free specific binding sites (type 2) is possible. The second common step in any immunoassay method is the formation of binding of the enzyme-labeled compound to a specific complex or free binding centers. Finally, the final mandatory process in immunoassay is the transformation of the enzyme tag into an appropriate signal measured by some physicochemical method (spectrophotometric, fluorimetric, fluorescent, etc.), which is achieved by measuring the rate of transformation of the substrate or the amount of product formed over a fixed time interval. Taking into account the above described approaches for the determination of specific complexes, further classification of immunoassay methods can be made according to the type of reagents used in the first stage of analysis. If at the first stage only the compound being analyzed and its corresponding binding centers (antigen and specific antibodies) are present in the system, the method is non-competitive. For a type 1 noncompetitive assay, the optimal ratio of components is one in which the concentration of

binding centers is significantly greater than the concentration of the compound to be determined. A prerequisite for the Type 2 non-competitive assay is that an excess or comparable concentration of the compound (antigen) to the specific binding sites is maintained, since the difference between the total number of binding sites and the number of immune complexes formed is determined. If the analyte compound and its analogue (enzyme-labeled analyte compound or analyte compound immobilized on a solid phase) are simultaneously present in the system at the first stage of analysis, competing for the specific binding centers available in relative shortage, the method is competitive. A prerequisite for the competitive method is a lack of specific binding centers relative to the total concentration of the analyzed compound and its analog. The next principle of classification of immunoassay methods is their division by the type of reactions performed at each of the immunochemical stages. In accordance with this, all methods can be divided into two groups - homogeneous and heterogeneous. Various variants of solid-phase immunoassay have now been developed:

1. "Sandwich" method. The general scheme of the method is as follows. Antibodies to the antigen under study are adsorbed on the solid phase. After incubation of the test material and formation of the antibody-antigen complex, the unbound components are removed and the conjugate, i.e. enzyme-labeled antibodies to the desired antigen, is added. At the end of incubation, followed by removal of unreacted conjugate by washing, a complex is formed in which the antigen seems to be enclosed between two layers of antibody. The presence of enzyme-labeled antibodies is detected using the appropriate substrate. "The sandwich method is used to detect HBsAg, HBeAg, hepatitis A virus antigen.

2. Indirect ELISA. Antigen is immobilized on a solid phase, after incubation of the test material and removal of unbound components, enzyme-labeled antibodies to human immunoglobulins of the IgG class are added, which interact with the Fc-fragment to IgG. After the substrate-enzymatic reaction, the results are recorded. In the presence of antibodies, the level of optical density of the reaction exceeds the values of negative samples. This method is used to detect antibodies to the hepatitis C virus.

3. Competitive method. To the antigen immobilized on the solid phase simultaneously add the test material and conjugate. During the reaction, the labeled and test antibodies compete for the active centers of the antigen immobilized on the solid phase. After completion of incubation and removal of unreacted components, an enzymatic reaction is carried out, the results of which are inversely proportional to the amount of antibody in the test sample.

4. Inhibitory ELISA. Standard AG is adsorbed on a polystyrene ball, after incubation with the test material and removal of unreacted components, enzyme-labeled AG is added, which interacts with free antibody binding centers that have interacted with the antigen sorbed on the solid phase. In the presence of antibodies in the test sample, the level of optical density of the reaction exceeds the values of negative control samples.

5. Direct ELISA. In the first step of the reaction, the test sample is fixed on a solid phase. The conjugate is then added to it. After removal of unreacted reaction components, an enzymatic reaction is carried out, the intensity of which is directly proportional to the content of the antigens under study in the sample and generally indicates their presence in the material under study. Competitive solid-phase methods are less sensitive than non-competitive methods. The limit of detection of various compounds for them is limited both by the sensitivity of enzyme label registration and antibody affinity, while for non-competitive methods, in the absence of nonspecific interactions, - only by the sensitivity of enzyme detection. Therefore, high-affinity antibodies must be used to achieve a high sensitivity of the competitive assay. Homogeneous ELISA Homogeneous ELISA methods are those that are performed in a single-phase system and do not require a stage of mechanical separation of the formed complexes. In all schemes of homogeneous immunoassay, the concentration of the remaining vacant specific binding sites, rather than the specific antibody-antigen complex formed, is recorded. However, in contrast to heterogeneous schemes, the observed enzymatic activity corresponding to the concentration of unoccupied specific binding sites can either decrease or increase due to the different nature of the effect of ligand binding on enzymatic activity.

Thus, the introduction of a tag into the antigen molecule is one of the most common approaches in homogeneous immunoassay methods. All homogeneous methods are competitive and are based on the simultaneous interaction of the analyzed and labeled antigens with antibodies. After the formation of the corresponding immunochemical complex in the solution, the enzymatic activity is measured, which is proportional to the concentration of free or bound labeled ligand. The antibody forming a complex with the antigen inhibits the activity of the bound enzyme.

References

1. Kudratova Z. E. et al. Modern etiology of anemias //Open Access Repository. - 2023. - T. 10. - №. 10. - C. 1-4.1. Burxanova D. S., Umarova T. A., Kudratova Z. E. Acute myocarditis linked to the administration of the COVID 19 vaccine //Central Asian Journal of Education and Innovation. - 2023. - T. 2. - №. 11. - C. 23-26.
2. Kudratova Z. E. et al. Atypical microflora etiologii li utkir obstructive bronchitislarining ў ziga hos klinik kechishi //Research Focus. - 2022. - T. 1. - №. 4. - C. 23-32.
3. Kudratova Z. E, Normurodov S. Etiological structure of acute obstructive bronchitis in children at the present stage - Thematics Journal of Microbiology, 2023. P.3-12.
4. Kudratova Z. E., Tuychiyeva S. K. Atipik mikroflora etiologiyali o'tkir obstruktiv bronxitlar etiopatogenezining zamonaviy jixatlari. Research Focus, 2023, B. 589-593.
5. Kudratova Z. E., Karimova L. A. Age-related features of the respiratory system. Research Focus,Tom 2, P. 586-588.
6. Isomadinova L. K., Daminov F. A. Modern laboratory diagnosis of chronic pyelonephritis in children // Journal of new century innovations. - 2024. - T. 49. - №. 2. - C. 112-116.
7. Isomadinova L. K., Daminov F. A. Glomerulonefrit kasalligida sitokinlar ahamiyati //Journal of new century innovations. – 2024. – T. 49. – №. 2. – C. 117-120.

8. Isomadinova L. K., Qudratova Z. E., Shamsiddinova D. K. Samarqand viloyatida urotiliaz kasalligi klinik-kechishining o'ziga xos xususiyatlari //Central Asian Journal of Education and Innovation. - 2023. - Т. 2. - №. 10. - С. 51-53.
9. Isomadinova L. K., Qudratova Z. E., Sh B. F. Virusli gepatit b fonida Covid-19 ning klinik laborator kechish xususiyatlari //Journal of new century innovations. - 2023. - Т. 30. - №. 3. - С. 60-65.
10. Isomadinova L. K., Yulayeva I. A. Buyraklar kasalliklarning zamonaviy diagnostikasi // Central Asian Journal of Education and Innovation. - 2023. - Т. 2. - №. 10 Part 3. - С. 36-39
11. Kudratova Z. E., Umarova T. A. Immunologic aspects of acute obstructive bronchitis in children //Science and Education. - 2023. - Т. 4. - №. 2. - С. 302-307.
12. Kudratova Z. E. et al. Hemoglobin indices in patients with anorectal malformations //Coloproctology. - 2017. - №. S3. - С. 27-27.
13. Jabbarova D., Karimova M. Improvement of diagnosis, treatment and prevention of hearing loss in children with otitis media //Theoretical aspects in the formation of pedagogical sciences. - 2024. - Т. 3. - №. 6. - С. 28-31.
14. Amonov Sh., Karimova M. Analysis of the results of complex diagnostics of toxoplasmosis and cytomegalovirus in children with hearing impairment //Models and methods in modern science. - 2024. - Т. 3. - №. 4. - С. 145-148.
15. Karimova M. M., Amonov Sh. E. Role of toxoplasmosis and cytomegalovirus infection in sensorineural hearing loss in children //Problems of postcovision otorhinolaryngology. - 2022. - С. 152-156.

Web of Discoveries: Journal of Analysis and Inventions
webofjournals.com/index.php/3